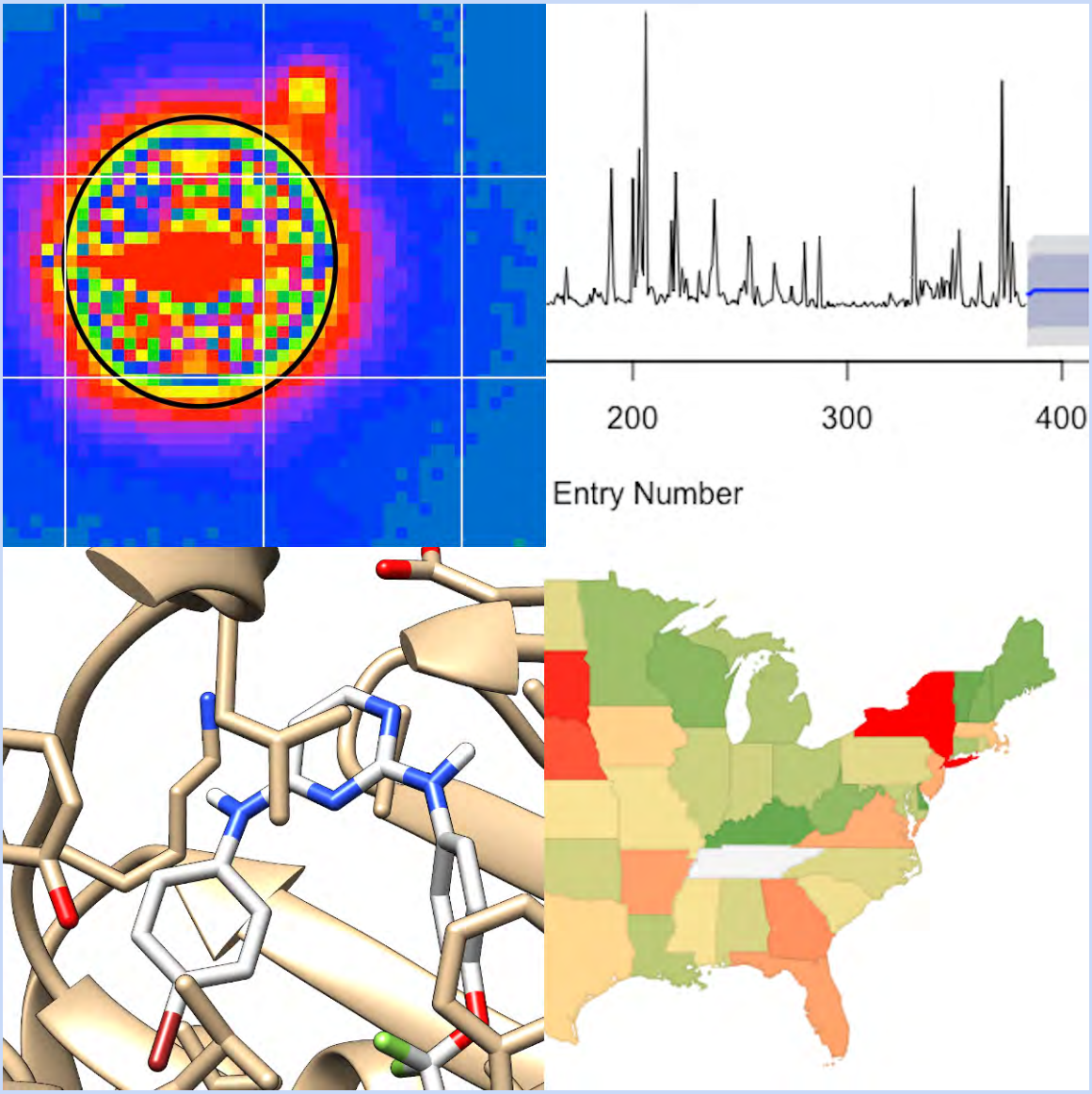
ASDRP Communications



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Preface | *ASDRP Communications*

Now in its third year, ASDRP is continuing to grow and mature. With the support of their advisors, over 350 students participated in a total of over 70 research projects across a diverse range of STEM fields—chemistry, environmental science, psychology, and more. The students worked hard, diving headfirst into the unfamiliar, discovering the challenges and rewards of research in just a single summer. This journal presents the fruits of their labor. I hope you enjoy reading about the astonishing science these students have produced.

— Avery Kruger, Editor-in-Chief

ASDRP Communications is a student-written journal that features research done in ASDRP by high school students. More information about the ASDRP program can be found online at www.asdrp.org.

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Comparison of Stress Responses Due to Various Salinity Amounts in Invasive (*Raphanus raphanistrum*) Versus Crop Radish (*Raphanus sativus L.*) Species

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Increasing salinity levels have shown to be detrimental to crop development as it imposes an oxidative stress in plants. However, as salinity has been increasing overtime, many plants have also become resistant to salt changes. This experiment focuses on the effects of different salt concentrations on an invasive versus crop radish species to test which of the two is more fit and resistant to withstand the Sodium Chloride (NaCl) addition to the soil. The experimental design consists of six pots for each wild radish and crop radish group. In each group, there are two controls, and the other four were treated with increasing concentrations of salt (0.25M, 0.50M, 0.75M, 1.00M). After repeating this process for two weeks, Ferrous oxidation-xylenol (FOX) Assay was conducted to quantify the hydrogen peroxide concentration present in the experimental groups. The results showed that the cherry radish plants had more rapidly increasing hydrogen peroxide levels as compared to the wild radish plant, meaning it is not fit to withstand salt stress. The wild radish plant was shown to be more fit and resistant when the salt was added to its soil.

domestic radish (*Raphanus sativus*) | Wild Radish (*Raphanus raphanistrum*) | oxidative stress | sodium chloride (NaCl) | cover crop | ferrous oxidation-xylenol (FOX) Assay

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Introduction

Soil salinity is a problem that farmers and agricultural researchers have been facing for many years, and the problem is only getting worse due to pollution. Most crops are sensitive to salt concentrations as it imposes an oxidative stress on the plants as additional salts in the soil may upset nutrient intake and balance of compounds in the plant systems. Therefore, yield percentages of many important crops have stooped to as low as 20%-50% as a result of the poor and unfavorable conditions to grow them[1].

When people think about radish farming, people most likely think about them as crops and for the purpose of harvesting. However, recent studies show that they have a wider use, and that they can be used for cover crop purposes. The phenotype of the radish is well suited to execute many valuable cover crop functions such as providing soil cover, scavenging nutrients, suppressing weeds, and alleviating compaction.

Cover crops are generally used to increase soil fertility, and to keep it intact in order to prepare the soil to reap a variety of different crops. It is an efficient and environmentally safe tool to manipulate microbiome composition in agricultural soils and has clear benefits in crop output[1]. As cover crops, radishes are typically used to aid farmers in the process of crop rotation, and the recycling of nutrients.

A systematic or recurrent sequence of crops grown over a number of cropping seasons is a common definition of crop rotation[2]. Radishes have several characteristics and attributes that allow them to pose as cover crops in farms. Firstly, their roots are able to grow almost three feet in sixty days. After the plant dies, the channels created by the roots tend to stay open at the soil surface, which improves infiltration, surface drainage, and soil warming[2,3] Radishes also undergo a process called bio-drilling which improves root growth by subsequent crops and access to subsoil.

The purpose of identifying various uses of radish plants is to formulate an experiment to stress out certain genes in radishes, and to design an experiment to measure the effect of oxidative stresses on a plant. Using this information, it is possible to test specific variables such as salinity on the phenotype or other direct effects of radish growth in either natural or domestic environments. Doing this can provide information about which plants are more resistant to changes in salt in the surrounding environment, and which crop would serve as a more effective cover crop.

Oxidative Stressors

Oxidative stressors are any external factor, whether biotic or abiotic, that impacts the oxygen levels in an organism. It results as an overproduction or accumulation of oxygen containing compounds which could be detrimental to the well-being of the organism, and may interfere with life processes due to an imbalance of nutrients. This experiment focuses on abiotic stresses of salt as they can be easily controlled and monitored in a laboratory environment. To test the effect of a stress on plants, Hydrogen Peroxide levels will be tested using the FOX Assay protocol[4,5].

Radish vs Wild Radish.

This experiment stresses out two types of radish species: Cherry Radish (Raphanus sativus) and Wild Radish (Raphanus raphanistrum), totest which makes a more effective cover crop based on its resistance to soil salinity

(NaCl). Wild radishes can grow to one and a half meters high and have tough, fibrous stems. They also have narrower roots than their domestic counterparts, although both can be eaten[2]. The wild radish is considered a noxious agricultural weed because it can become a host for pests and diseases, in addition to making harvesting difficult by choking header combs. Wild radishes are also on rare occasion toxic to livestock. Domestically grown radishes, however, may take over a month to flower while wild radishes only take a couple weeks to sprout.

Methods

Materials

To conduct the research, seeds of two different Radish species, Raphanus raphanistrum and Raphunis Sativus were purchased. Raphanus raphanistrum seeds were from the brand Seeds of Change, and Raphanus sativus seeds were purchased from the Nature's Seeds brand. These plants were grown in Scotts Fertilizer, and indoor LED lights were used as a light source. For the FOX Assay protocol, the following chemicals were used: 12M perchloric acid, ammonium ferrous sulfate, 18M sulfuric acid, xylenol orange, and sorbitol. Additionally, the protocol required the use of a centrifuge, shaker, and spectrophotometer.

Setup

Simple methods were conducted for the project. As aforementioned, the objective of this experiment is to simulate an experiment to observe how stressing out specific genes in radishes affects said radishes. The salinity stress was used as it is an increasing problem to farmers trying to grow their crops in many different areas around the world. This experiment was set up with different groups with varying concentrations of water and brine solutions. The plants were first separated into two groups which were cherry and wild radishes. Next, they were separated into different widths of the plants. They were 0 m , 0.25 m , 0.5m, 0.75 m, and the last one is 1.0 m. After that, each of the plants had their own amounts of NaCl salt administered to them with tap water. For the 0m plant, there was no amount of salt added to the water. The 0.25m plant had been watered with 1.1g salt. The 0.5m plant had been fed an amount of salt which was 2.19g with its water. The 0.75m plant had around 3.29g of salt in its water. The 1m plant's amount of salt with the water was 4.38g. This procedure was repeated for about two and a half weeks.

FOX Assay

The FOX Assay was used to quantify the presence of Hydrogen Peroxide (H2O2) in the artificial lab setup. The extraction solution was 1.7 mL of 12 M perchloric acid (HClO4) diluted with 98.3 mL of deionized water, which was mixed into 0.2 grams of leaf parts. This solution was placed in the centrifuge for fifteen minutes, and 1.5 mL of the supernatant was added to 1.5 mL of the working reagent solution which consisted of 19.6 mg ammonium ferrous sulfate, 0.28 mL of 18M sulfuric acid, 14.3 mg xylenol orange (tetrasodium salt), and 3.64 g D-sorbitol. This solution was left to develop at 30 C for 30 minutes. Lastly, the test samples were put under a spectrophotometer to detect the absorbance of the solution at 560nm. Figure 1 shows levels which were then calculated in µmol/g using the equation extinction coefficient 2.24 x 105 M-1cm-1.

NaCl (M)	Wild		Cherry	
	Absorbance (AU)	H_2O_2 (umol/g)	Absorbance (AU)	H_2O_2 (umol/g)
0 M	0.95 + 0.01	$4.2x10^{-6}$	0.91 + 0.01	$4.06x10^{-6}$
.25 M	0.83	$3.71x10^{-6}$	1.00	$4.46x10^{-6}$
.50 M	1.04	$4.64x10^{-6}$	1.21	$5.40x10^{-6}$
.75 M	1.70	$7.59x10^{-6}$	1.79	$7.99x10^{-6}$
1.00M	1.50	$6.70x10^{-6}$	1.89	$8.44x10^{-6}$

Fig. 1. Hydrogen peroxide concentrations calculated by dividing absorbance value by extinction coefficient. (Extinction coefficient = 2.24 x 105 M-1cm-1)

Results

Absorbance values from the spectrophotometer only provided the information of absorbance of 560nm light. In order to obtain concentration of hydrogen peroxide, the Beer-Lambert law shown in Figure 2. was used to convert the absorbance values to Hydrogen Peroxide concentration.

$$A = \varepsilon c l$$

Fig. 2. Beer-Lambert Law. A=absorbance; ε=molar absorption coefficient, c= molar concentration, l= optical path length.

The hydrogen peroxide values were shown to be directly proportional to the increasing salt concentration, as expected. This means that is leading to an upset of compounds

in the plant which is due to the increased uptake of salt that is harmful to the plant. However, data shows that the wild radish species is more resistant to changes in NaCl concentration as the increase of hydrogen peroxide was more gradual as compared to the cherry radish as shown in Figure 3. This result was seen in the phenotypes of the plants before the FOX Assay was conducted, as the 0.75 and 1.0 M treated cherry radish plants were almost completely withered away as the same groups of wild radishes still had a few sprouts left.

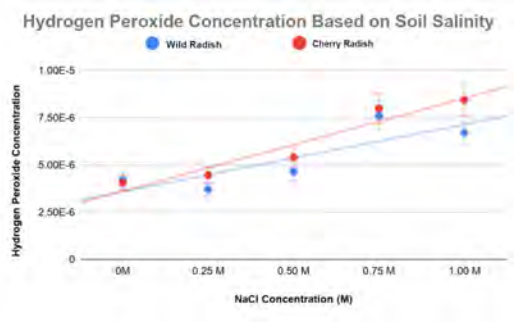

Fig. 3. Graph of H_2O_2 concentration relative to NaCl concentration.

Conclusion

If the plants were to be used as a cover crop, wild radish would be the most effective since it is more resistant to the salinity stress in its environment, and is able to grow bigger and stronger roots to keep the soil intact as compared to the cherry radish, in which the nutrient balance is easily upset with the added stress.

Cover cropping however, is not just limited to radish crops. Although this experiment showed the salt stress effect on an invasive versus domestically grown radish species, many more possibilities of cover crops exist, some of which may withstand a similar stress in a more effective way. This is due to the addition of other environmental factors such as temperature, heat, humidity, fertilizer use, etc, which may cause a different yield of results in a real life farm setting.

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Bibliography

1. John M DeLong, Robert K Prange, D Mark Hodges, Charles F Forney, M Conny Bishop, and Michael Quilliam. Using a modified ferrous oxidation- xylenol orange (fox) assay for detection of lipid hydroperoxides in plant tissue. *Journal of Agricultural and Food Chemistry*, 50(2): 248–254, 2002.
2. Yuki Mitsui, Michihiko Shimomura, Kenji Komatsu, Nobukazu Namiki, Mari Shibata-Hatta, Misaki Imai, Yuichi Katayose, Yoshiyuki Mukai, Hiroyuki Kanamori, Kanako Kurita, et al. The radish genome and comprehensive gene expression profile of tuberous root formation and development. *Scientific reports*, 5(1):1–14, 2015.
3. Jungeun Lee and Ilha Lee. Regulation and function of soc1, a flowering pathway integrator. *Journal of experimental botany*, 61(9):2247–2254, 2010.
4. Caroline E Ridley and Norman C Ellstrand. Evolution of enhanced reproduction in the hybrid-derived invasive, california wild radish (raphanus sativus). *Biological Invasions*, 11(10):2251, 2009.
5. Jinglei Wang, Yang Qiu, Feng Cheng, Xiaohua Chen, Xiaohui Zhang, Haiping Wang, Jiangping Song, Mengmeng Duan, Haohui Yang, and Xixiang Li. Genome-wide identification, characterization, and evolutionary analysis of flowering genes in radish (raphanus sativus l.). *BMC genomics*, 18(1):981, 2017.